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Chang K, Ding I, Kern FG, Willingham MC.

Ultrastructural Cytochemistry Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

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Brief Report

Immunohistochemical Analysis of p53 and HER-2/neu Proteins in Human Tumors[1]

KAI CHANG,[2] IVAN DING, FRANCIS G. KERN, and MARK C. WILLINGHAM

Ultrastructural Cytochemistry Section, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892 (KC,MCW), and Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC 20007 (ID,FGK).

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We examined samples of tumors of human breast, ovary, and colon of various degrees of malignancy for the expression of p53 protein, using a panel of anti-p53 antibodies and peroxidase immunohistochemistry. Of 66 tumor cases (24 cases of ovarian carcinoma, 23 cases of colon adenocarcinoma, and 19 cases of breast carcinoma), 36 (53%) showed high levels of expression of p53 using a human-specific antibody, and 16 (24%) showed high expression of a mutant form of p53. In the mutant p53-positive breast tumor samples, six (86%) were positive for HER-2/neu reactivity, compared with colon (0/4) and ovarian tumors (1/5). The pattern of p53 intracellular localization and tissue distribution, and the relationship between the expression of mutant p53 and cell differentiation, were also examined; poorly differentiated cells showed either overexpression of p53 or higher levels of mutant p53 in comparison with more normal cells. (*J Histochem Cytochem 39:1281–1287, 1991*)

KEY WORDS: p53; HER-2/neu; Immunohistochemistry; Peroxidase; Human breast cancer; Colon cancer; Ovarian cancer.

Introduction

p53 protein was first identified as a host cell protein bound to T antigen, the dominant transforming oncogene of the DNA tumor virus SV40 (20). Normal p53 is thought to act as a tumor suppressor gene in a murine model and human systems, but various point mutations within the coding region of the murine gene can convert it from a recessive suppressor gene to a dominant oncogene (11). In support of this theory, it has recently been shown in transfection experiments that p53 clones that transform are all mutants (17) and that wild type p53 suppresses transformation (2,7,10,11,22).

Interest in p53 was greatly stimulated by the finding of abnormalities of the gene and its protein in tumors of human colon (24,30), breast (4,6,25), lung (9,28), and in other malignant tissues (8,23,26). The human p53 gene is on the short arm of chromosome 17, a frequent site of allele loss in common human cancers (1,19,21). Direct sequencing of p53 from various types of tumors and tumor cell lines suggests that p53 mutation is a very common event in human carcinogenesis (5,8,9,18,23,24,26,28). The mutations that are detected in human and murine tumors and cell lines are mostly missense point mutations in highly conserved amino acids (12,24,28) which are mainly found in five conserved regions, although recently Takahashi et al. (27) reported that there is an intronic point mutation of p53 in human lung cancer.

The analysis of expression of both normal and mutant p53 and other oncogene products in human primary cancer tissues is increasingly important in gaining a better understanding of the process of tumorigenesis and in the search for these markers for the early diagnosis of malignant transformation. In this article we describe an immunohistochemical analysis of the expression of p53 and HER-2/neu proteins in cancers of human breast, ovary, and colon and in normal tissues, and discuss the relationship of the expression of these oncogene products with a phenotypically distinct subset of cancer tissues.

Materials and Methods

Human tumors and Normal Tissues. Tissues for immunohistochemical analysis, both malignant and non-malignant, were obtained from the National Disease Research Interchange (NDRI; Philadelphia, PA), and were immediately snap-frozen in liquid nitrogen before being stored in a −80°C freezer. Non-malignant tissues from either cancer patients or normal subjects were bladder, brain, skin, prostate, liver, and kidney. Each tissue block, including four to six tumor cases, was embedded in OTC (Miles; Elkhart, IN) and stored at −70°C before sectioning.

Monoclonal Antibodies. Monoclonal antibody (MAb) Ab1 (PAb421), specific to mammalian p53, recognizes an epitope located between amino acids 370–378 of p53 (15). Ab2 (PAb1801) is a human-specific antibody that recognizes an epitope in p53 between amino acids 32–79 (3). Ab1 and Ab2 detect both normal and mutant forms of p53. Ab3 (PAb240), specific to mutant p53, recognizes a denaturation-resistant epitope located between amino acids 156–335 (13). These three antibodies and anti-HER-

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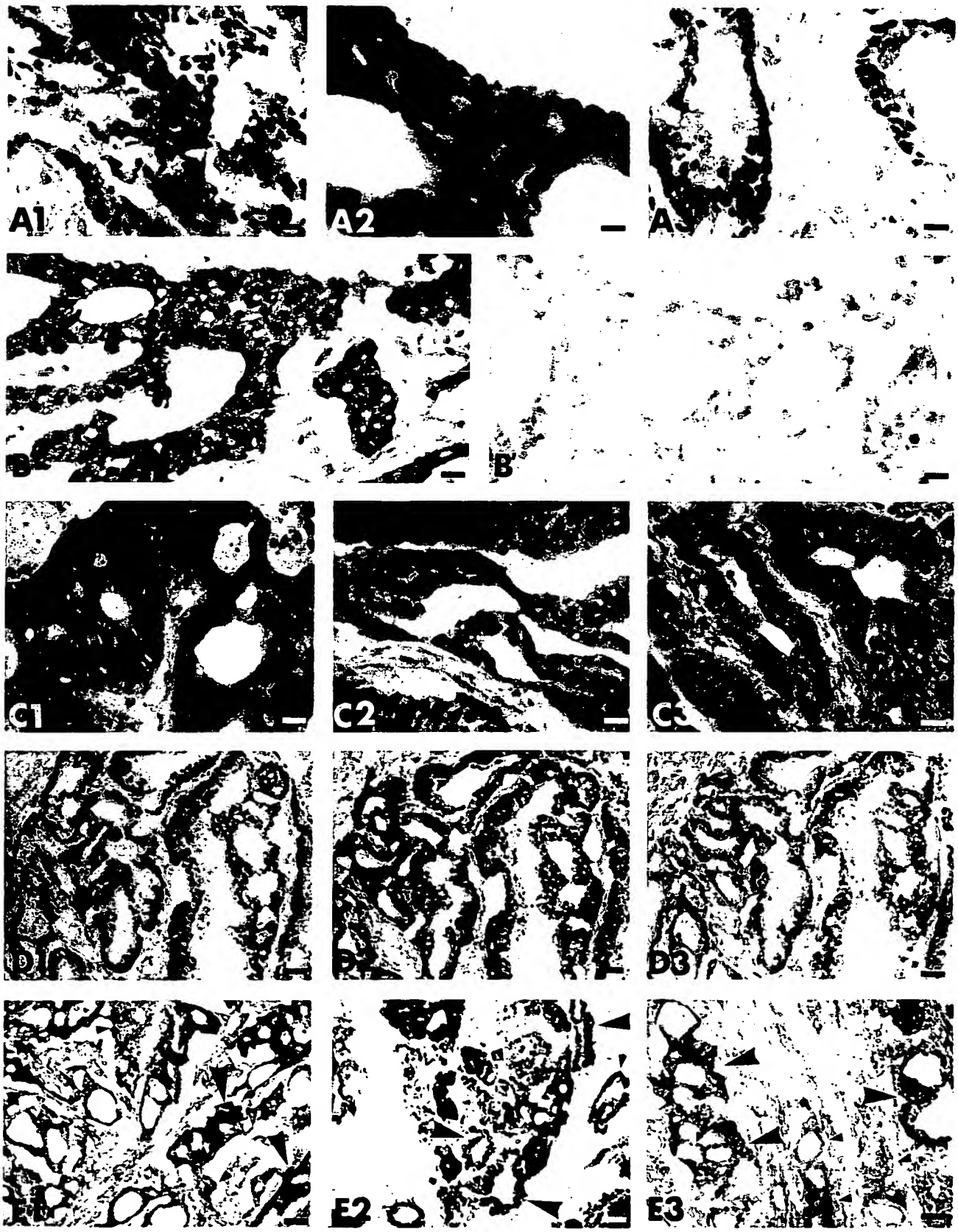
[2] To whom correspondence should be addressed.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2/neu antibodiy, which reacts with the extracellular domain of the HER-2/neu protein, were purchased from Oncogene Science (Manhasset, NY) and all antibodies used in experiments were diluted from the concentrated stocks and stored at −20°C in a buffer solution containing 1 mg/ml carrier protein (bovine serum albumin or normal goat globulin). HB21, an anti-transferrin receptor antibody (American Type Culture Collection; Rockville, MD), and J6, a mouse anti-rat disulfide isomerase species-specific antibody (14), were used as positive and negative controls, respectively.

Peroxidase Immunohistochemistry. Tissue sections were cut using a cryostat (Hacker Instruments; Fairfield, NJ) at 6-μm thickness and were thaw-mounted on Histostik (Accurate Chemical; Westbury, NY)-coated coverslips, allowed to air-dry, and then lyophilized for 4 hr. All subsequent steps were performed at room temperature. The sections were then fixed for 10 min in 100% acetone and dried, then rehydrated in PBS for at least 10 min. Tissues were then incubated in 10% normal goat serum in PBS for 10 min, followed by washing in PBS. Sections were incubated for 1 hr with the primary mouse MAb Ab1, HB21, or J-6 at a concentration of 10 μg/ml, or Ab2 and Ab3 at 3 μg/ml, diluted in 4 mg/ml normal goat globulin, 0.1% saponin (Sigma; St Louis, MO), 1 mM EGTA (Sigma) with PBS (NGG-Sap-PBS). After washing in PBS, samples were incubated with 25 μg/ml affinity-purified horseradish peroxidase-conjugated goat anti-mouse IgG (H+L) (Jackson Immuno-Research; Avondale, PA) in NGG-Sap-PBS with 5% human AB serum. Sections were washed in PBS, followed by a 10-min incubation in NGG-Sap-PBS, and further washing in PBS. Peroxidase was visualized by reaction with 0.3 mg/ml diaminobenzidine (Sigma) and 0.01% H_2O_2 in PBS for 10 min. Light counterstaining was carried out with Gill's hematoxylin for 2 min, following by washing in PBS, then 1% OsO_4 in H_2O for 1 min. Sections were dehydrated in sequential ethanol and xylene and mounted in Permount (Fisher Chemical; Fairlawn, NJ). All slides were reviewed by two individuals independently.

Immunohistochemical Evaluation. Immunohistochemical analysis of each case, using three anti-p53 antibodies and anti-HER-2/neu antibody, was based not only on the percentage but also on the intensity of positive staining, as well as several pathological parameters, such as histological grading and differentiation, with special emphasis on characterization of p53 intracellular staining and tissue distribution patterns. A "homogeneous" pattern of p53 tissue distribution was used to define those p53-positive samples in which p53 expression was distributed in all malignant cells of the samples tested. A "heterogeneous" pattern represents a p53 distribution characterized by expression in selected foci containing many cells in an otherwise negative area of tumor. Both patterns were further characterized as "variable" reactivity in which each positive cell had a similar degree of label. A very rare pattern was also found, even though it may not be recognized as a positive case owing to its very low percentage of reactivity (<3%); this is classified as a "sporadic" pattern in which a few strongly positive cells were individually distributed throughout the tumor sample. All of the tissue distribution types listed above refer to either a nuclear pattern, a cytoplasmic pattern, or a nuclear pattern with weak cytoplasmic reactivity.

Results

Patterns of p53 Intracellular Localization and Tissue Distribution in Human Breast, Colon, and Ovarian Carcinomas

p53 protein was usually present in the nucleus of positive cells with or without weak cytoplasmic reactivity, using all three antibodies to p53 (Figure 1). In this study, we divided p53 localization into three categories as listed in table 1. Among the three types of carcinoma, all three antibodies showed major reactivity of p53 in the nucleus, either in the nucleus alone or in the nucleus with a weak cytoplasmic signal. Cytoplasmic expression of p53 was not observed with Ab1 (0/21) and Ab3 (0/16), whereas 30% of Ab2-positive cases (11/36) were found to have a weak reaction in the cytoplasm. Five patterns of p53 tissue distribution were also classified: a homogeneous pattern with variable or uniform staining, a heterogeneous pattern with variable or uniform staining and, very rarely, a sporadic pattern with rare single cells labeled. This latter pattern was not counted as p53 positive owing to the low percentage of positivity (<3%). As shown in Table 2, p53 localization in a homogeneous pattern was the most common with Ab2 (80%) and the least common with Ab1 (38%), whereas Ab3 was in between (50%). Even though both Ab1 and Ab3 showed remarkable focally positive distributions in some tumor samples, Ab1 tended to be more homogeneous within each tumor compared with Ab3.

Table 1. *p53 intracellular localization patterns*

	Breast cancer (n = 19)			Colon cancer (n = 23)			Ovarian cancer (n = 24)			All cancers (n = 66)		
Patterns	Ab1	Ab2	Ab3	Ab1	Ab2	Ab3	Ab1	Ab2	Ab3	Ab1	Ab2	Ab3
Nucleus	3	0	3	5	0	1	3	4	2	11	4	6
Cytoplasm	0	1	0	0	4	0	0	6	0	0	11	0
Nucleus and cytoplasm	1	10	4	1	10	3	8	1	3	10	21	10
Total	4	11	7	6	14	4	11	11	5	21	36	16

Figure 1. Immunohistochemical localization of p53 in cryostat sections of human tumors. Peroxidase immunohistochemistry was performed as described in Materials and Methods. Three murine MAb (Ab1, Ab2, and Ab3) were used to localize p53 in breast, colon, and ovarian carcinomas, and three forms of p53 intracellular staining patterns were observed: a nuclear pattern (**A1** for Ab1, **A2** for Ab2, and **A3** for Ab3, all in colon carcinoma), a cytoplasmic pattern (**B** for Ab2), and a nuclear and cytoplasmic pattern (**C1** for Ab1, **C2** for Ab2, and **C3** for Ab3, all in colon carcinoma). Two major tissue distribution patterns are also shown as a homogeneous pattern (**D1** for Ab1, **D2** for Ab2, and **D3** for Ab3 in the same colon carcinoma) and a heterogeneous pattern (**E2** for Ab2 in ovarian carcinoma, **E1** for Ab1, and **E3** for Ab3 in colon carcinomas). Large arrowheads mark positive foci, and small arrowheads mark negative areas. All samples processed using a negative control MAb, J6, in place of the p53 MAb showed no specific reactivity (an example shown in **B** in breast carcinoma). Original magnifications: **A–C** × 200; **A2** × 400; **D,E** × 68. Bars: **A–C** = 20 μm; **A2** = 10 μm; **D,E** = 59 μm.

Table 2. *p53 tissue distribution patterns*

Patterns[a]	Breast cancer			Colon cancer			Ovarian cancer			All cancers		
	Ab1	Ab2	Ab3	Ab1	Ab2	Ab3	Ab1	Ab2	Ab3	Ab1	Ab2	Ab3
Homog. pattern, uniform intensity	0	6	2	4	11	3	2	7	3	6	24	8
Homog. pattern, variable intensity	1	3	0	0	1	0	1	1	0	2	5	0
Heterog. pattern, uniform intensity	1	0	0	2	2	0	8	3	1	11	5	1
Heterog. pattern, variable intensity	2	2	5	0	0	1	0	0	1	2	2	7
Total	4	11	7	6	14	4	11	11	5	21	36	16

[a] The sporadic pattern is rare and its positive content of cells is usually less than 3%; this pattern was not included in the p53-positive group in this study.

Detection of p53 Overexpression in Breast, Ovarian, and Colon Tumors

Three different anti-p53 MAb (Ab1, Ab2, Ab3) were used to examine a total of 66 tumor samples (24 cases of ovarian cancer, 23 cases of colon adenocarcinoma, and 19 cases of breast cancers). Of the 66 tumor samples, 21 cases (32%) were positive with Ab1, 36 cases (55%) with Ab2, and 16 cases (24%) with Ab3. The normal tissues adjacent to tumor, which showed no histological evidence of malignant invasion, were negative with all of these three antibodies. In three types of tumor, Ab2 showed the highest percentage of cases of positive labeling compared with Ab1 and Ab3 (Table 3). When Ab3 was used as a specific antibody to detect the mutant form of p53, there were only a small number of positive colon (17%) and ovarian tumor samples (21%), but mutant p53 protein product was detected at a higher frequency in breast tumors (37%). However, Ab1, an MAb against p53 from multiple species, had a higher expression in 24 cases of ovarian tumor (46%) compared with the expression in breast and colon tumors (21% and 26%).

Co-overexpression of p53 and HER-2/neu in Breast Tumors but Not in Ovarian and Colon Tumors

Among 23 colon and 24 ovarian tumors, HER-2/neu protein expression was relatively low compared with its expression in breast tumors. Of the 19 primary breast tumors, eight (42%) showed a very strong positivity with an MAb against HER-2/neu (Figure 2). Most of the HER-2/neu-positive cases were non-infiltrating or infiltrating ductal adenocarcinoma. The other histopathological types of breast tumors were usually negative or weak for HER-2/neu staining. More striking was that the breast tumor samples with high expression of HER-2/neu also had an overexpression of p53 protein, especially with Ab2 and Ab3 (Table 4). Six out of seven cases of Ab3-positive breast tumors showed a HER-2/neu-positive pattern with a plasma membrane distribution. Only one Ab3-positive case did not fit this relation of p53 and HER-2/neu coexpression; this sample was a squamous cell carcinoma, a totally different histopathological type of breast cancer.

Table 3. *p53 expression in three types of human carcinomas*

Carcinoma tested	p53 Ab1		p53 Ab2		p53 Ab3	
	No.	%	No.	%	No.	%
Breast (19)	4	21	11	58	7	37
Colon (23)	6	26	14	61	4	17
Ovary (24)	11	46	11	46	5	21
Total (66)	21	32	36	55	16	24

Correlation of Mutant p53 Overexpression with Poorly Differentiated Tumors

The frequency of expression of the mutant form of p53 detected with Ab3 was not as high as the normal or normal–mutant complex form of p53 detected by Ab1 or Ab2 in these different types of tumors. Although mutant p53 reaction showed mostly a heterogeneous distribution with variable intensity in breast tumors, and a homogeneous distribution with uniform intensity in colon and ovarian tumors, the expression of mutant p53 protein seemed to correlate with poor differentiation and cell proliferation. The Ab3-positive staining cells often had bizarre nuclei or other abnormal morphological features (Figure 3). In some poorly differentiated tumor samples, the malignant cells with denser nuclear staining, or cells with a small compact nucleus, often had a strong Ab3-positive reaction in the nucleus (Figure 3B).

In the normal colon mucosa no positive staining could be seen when these three MAb were used, but in highly proliferative areas of colon epithelium, such as severe hyperplasia and moderate dysplasia, there was a weakly positive reaction with these antibodies; most of these cases showed a homogeneous and uniform cellular labeling pattern. On the other hand, stronger localization of mutant p53 was observed in the areas containing the most poorly differentiated tumor cells (results not shown). In contrast, HER-2/neu expression, which perhaps occurs at an earlier stage of transformation, was moderately positive in hyperplastic epithelium and completely negative in poorly differentiated foci (results not shown).

p53 Expression: Only in Malignant Tumors and Uncommon in Normal Tissues

In all of the tumor samples in this study, normal epithelium adjacent to malignancy did not appear to react with any of the antibodies against p53. Some areas with hyperplastic or slightly dysplastic epithelium showed moderately positive staining with Ab1 and/or Ab2. The malignant tissues with positive staining using Ab3 showed variations in the intensity and distribution of labeling in different types of tumor samples, in addition to the positive staining with either Ab1 or Ab2. In the p53-positive areas, labeled cells appeared to be either uniform or variable with a nuclear and/or

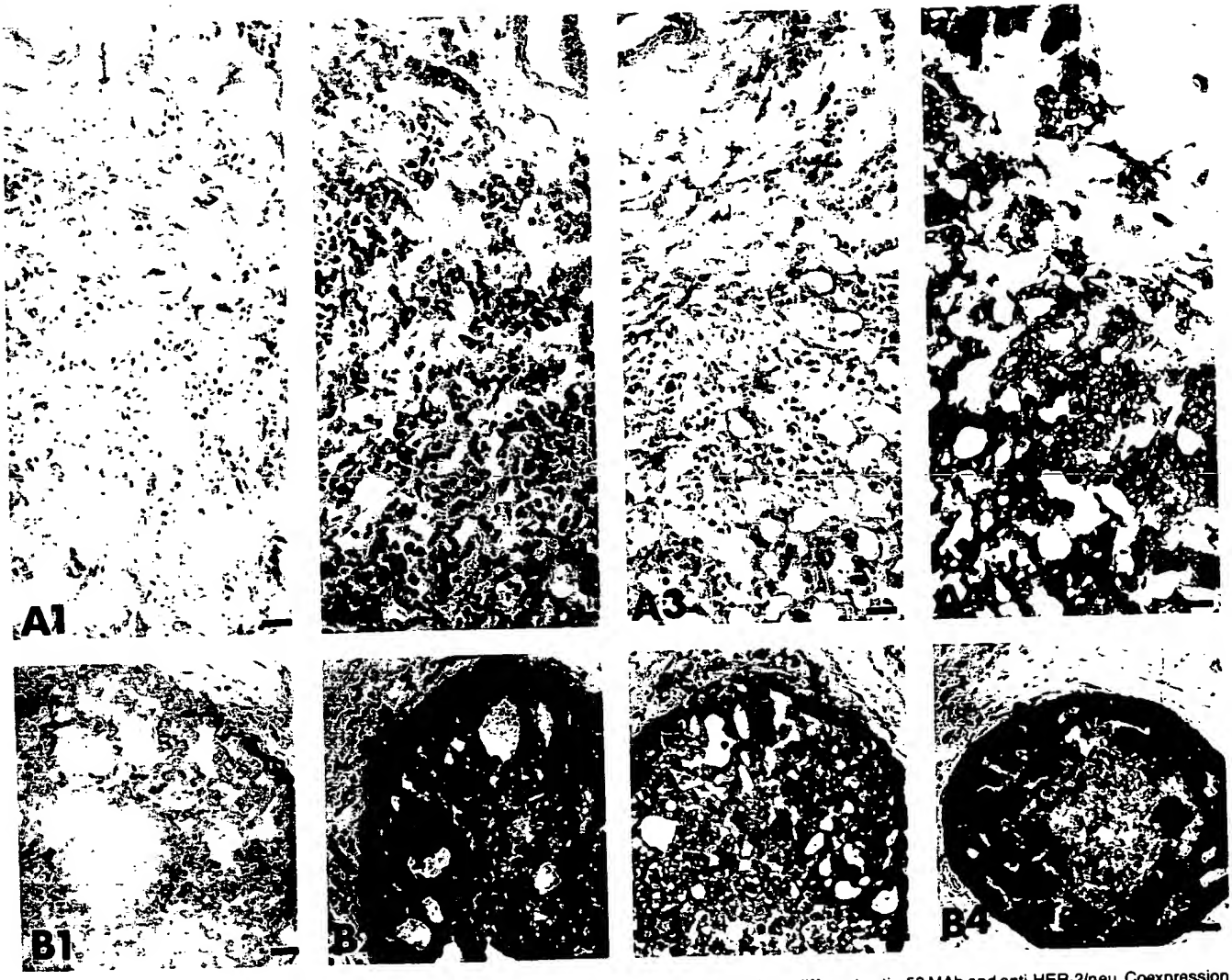

Figure 2. Immunoperoxidase localization of p53 and erbB2 (HER-2/neu) in breast carcinoma using three different anti-p53 MAb and anti-HER-2/neu. Coexpression of p53 and HER-2/neu is shown (A) in a poorly differentiated breast carcinoma and (B) in an infiltrating ductal carcinoma, in which 1, 2, and 3 show results with Ab1, Ab2, and Ab3, and 4 shows the result with anti-HER-2/neu. A was the only case tested that showed reactivity with all three anti-p53 antibodies; b represents most of the coexpression cases in which Ab1 failed to react. Note that p53 expression in case A is in a homogeneous, nuclear pattern only, whereas in case B the localization is mainly in a homogeneous, but nuclear *and* cytoplasmic pattern. Original magnification × 100. Bars = 40 μm.

cytoplasmic staining pattern. No correlation could be drawn between the expression of mutant p53 and a specific histopathological type of tumor because of the small number of cases tested in this study. In most normal tissues (stomach, colon, bladder, brain, prostate, and muscle), no p53 localization was found, but in some normal tissues cells with a potential proliferative ability showed reaction with p53 antibodies, such as the basal cells in esophageal epithelium and skin. A more detailed study of the expression of p53 in normal tissues is in progress.

Table 4. *HER-2/neu and p53 coexpression in human breast carcinomas*

	p53 Ab1		p53 Ab2		p53 Ab3	
	-	+	-	+	-	+
HER-2/neu pos.	7	1	0	8	2	6
HER-2/neu neg.	8	3	8	3	10	1
Total	15	4	8	11	12	7

Discussion

This study shows that increased expression of p53 at the protein level occurs with high frequency in the three types of tumors examined (breast, colon, and ovary). Several methods have been used to assess p53 expression in human tumors. Assessing gene expression at the protein level may have certain adavantages. Mutation does not necessarily lead to any change in mRNA expression or molecular weight of mRNA. Therefore, antibodies suitable for screening primary tumors, particularly those which can detect epitopes expressed by mutant p53, could provide valuable information. Previous results from another group (6) showed positivity in

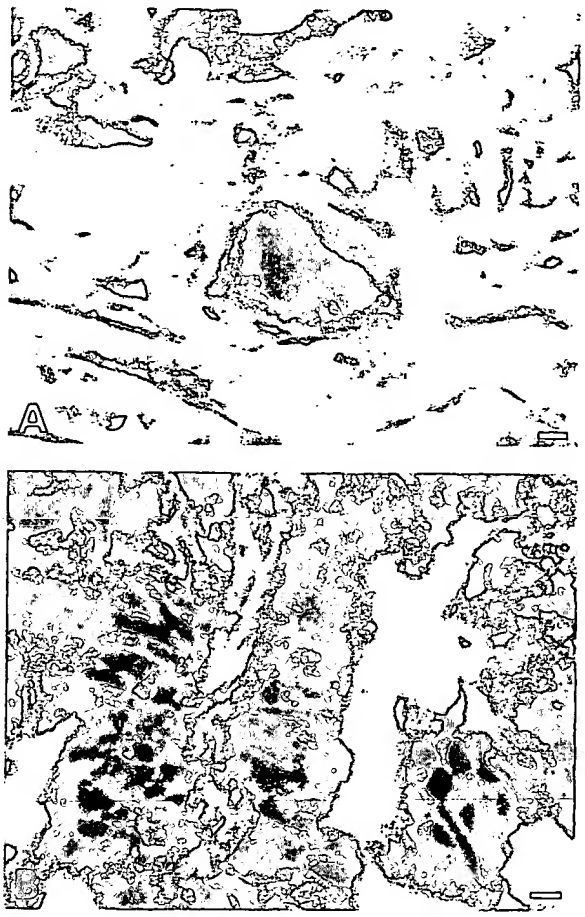

Figure 3. Examples of morphologically atypical cells showing mutant p53 expression. The expression of the mutant type of p53 was usually found in the carcinomas with poorly differentiated nuclei, as characterized by pleomorphism, hyperchromatism, mitotic figures, and irregularity of size, shape, and staining. (A) An atypical breast cancer cell with two nuclei stained positively for p53 using AB3. (B) The irregular nuclei of a colon adenocarcinoma labeled with Ab3. The negative control (J6) showed no reactivity with either of these samples (results not shown). Original magnification × 400. Bars = 10 μm.

nuclei of malignant cells in 15.5% (31 cases) of 200 human primary breast cancers using Ab1 (pAb 421), and 45.5% (88 cases) of breast tumor samples when Ab2 was used. In addition, Van Den Berg et al. (30) reported that p53 expression was detectable in 55% of 29 cases of colon carcinoma using Ab1. Our results from breast tumor samples are consistent with previous results (6), but the lower percentage of positive cases we have found in colon cancer (26%) compared with previous results (30) may be due to the different methods and/or different stages of tumor sampling. However, all studies showed that most of the localization of p53 is mainly found in the nuclei of malignant cells, even though there is light or moderate expression in p53 in some dysplastic tissues. Normal tissues examined either adjacent to malignancy or from normal subjects did not react with any of p53 antibodies.

Of the colon and breast carcinomas, 21% and 26%, respectively, were positive for Ab1, whereas an additional 37% and 35% of the breast or colon cases, respectively, showed reactivity with Ab2. The reason for this discrepancy is not clear; however, it may be attributable to the high specificity of Ab2. This difference was not observed in ovarian carcinomas. Thirty-seven percent of breast tumors showed reactivity with the highly specific antibody (Ab3), but a much lower rate of reaction was found in colon and ovarian tumors using Ab3.

On the basis of the putative role of p53 and its possible late expression in tumor development (16), it can be anticipated that p53 could be a marker for rapid proliferation, poor differentiation, or may be associated with bad prognostic factors. Our data showed that in breast carcinoma the positive cases for the mutant form of p53 also had a high expression of HER-2/neu oncogene product, and that in colon carcinoma mutant p53 distribution always correlated with poorly differentiated cells or cells with proliferative potential. In breast cancers, an association of p53 expression with estrogen receptor-negative, EGF receptor-positive tumors has been reported using either Ab1 and/or Ab2 and by assessing mRNA levels (6,29). In addition, Harris (16) recently reported the association of p53 expression, detected by AB3, with EGF receptor and HER-2/neu expression. Whether mutant p53 expression is a strong indicator for poor prognosis still requires analysis of a large number of cases for which detailed follow-up information is available.

By using a similar MAb against mutant p53, Iggo et al. (18) recently analyzed mutant p53 expression in human primary lung carcinomas and found the expression in 28 out of 40 lung carcinomas, 82% of squamous cell carcinomas showing abnormal p53 expression. In the panel of three types of human tumors reported here, we found only 37% of breast tumors that overexpressed this mutant form of p53. In the same experiments, a lower frequency of mutant p53 expression (17% and 21%) was detected in colon and ovarian tumors, respectively. In other studies, a group of human squamous cell carcinomas of esophagus and cervix showed a 30% frequency of p53 reaction with Ab3 (results not shown). Many factors other than the actual amount of protein may explain this discrepancy, such as different histological types of tumor and differing affinity of the antibodies used.

Our study suggests that P53 is likely to be a proto-oncogene that commonly undergoes mutation in human tumors, particularly in breast tumors. Because p53 mutations are not restricted to a single site in the gene, immunohistochemistry may well be the most straightforward means to identify p53 mutations in primary human tumors when the specific antibody for mutant p53 is available. The high frequency of p53 mutations in human cancers and our increasing understanding of this relationship may allow the development of new diagnostic and therapeutic agents. Obviously, Ab2 and Ab3 are very powerful reagents for the detection of p53 overexpression and mutations in breast cancer as well as in other malignancies.

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D'Emilia J, Bulovas K, D'Ercole K, Wolf B, Steele G Jr, Summerhayes IC.

New England Deaconess Hospital, Department of Surgery, Boston, Massachusetts 02115.

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Expression of the c-erbB-2 gene product (p185) at different stages of neoplastic progression in the colon

J. D'Emilia, K..Bulovas, K. D'Ercole, B. Wolf, G. Steele Jr. & I.C. Summerhayes

New England Deaconess Hospital, Laboratory of Cancer Biology, Department of Surgery, 50 Binney Street, Boston, Massachusetts, USA

The c-*erb*B-2 gene has been found amplified in a number of human adenocarcinomas leading to elevated levels of expression of the p185 protein product. Increased expression of this putative growth factor receptor has been reported to occur by molecular mechanism other than gene amplification and for this reason we have studied the expression of the p185 protein in normal colon and in lesions representing different stages of neoplastic progression. We report amplification of the c-*erb*B-2 gene in 3 of 44 colon carcinomas and 1 of 5 preneoplastic polyps studied. Confirmation of expression of the p185 protein product was established in Western blot analysis and by immunocytochemical staining of tissue sections. An extended study, involving adenomatous polyps and carcinomatous material in immunostaining, revealed detectable presence of the p185 protein in 20% of carcinomas, consistent with immunoprecipitation data derived using established cell lines. In contrast, a high percentage of polyps showed strong staining with both p185 antibodies used, indicating elevated levels of expression of the c-erbB-2 protein associated with preneoplastic lesions. Staining of normal human colon revealed a restricted localization of this putative receptor to cells on the luminal colonic surface, with no expression in cells of the crypt. Histologically normal mucosa, adjacent to the tumor, showed a more extensive distribution involving the crypt suggestive of a disturbance in the normal expression of c-*erb*B-2. These results indicate that elevated expression of the c-erbB-2 protein is associated with early stages of colonic neoplasia but do not establish it as a primary factor in these events. The occurrence of multiple copies of the c-*erb*B-2 in a percentage of colon lesions, however, suggests a possible role for this gene in some colon malignancies.

Introduction

The *neu* oncogene was first characterized in transfection assays using DNA isolated from rat neuro/glioblastoma cell lines (Shih *et al.*, 1981). Subsequent isolation of the gene (Hung *et al.*, 1986) and its human homologue (Semba *et al.*, 1985; Yamamoto *et al.*, 1986; Coussens *et al.*, 1985) has revealed a putative growth factor receptor, closely related to the EGF receptor (Schechter *et al.*, 1984), encoding a 185 kilodalton membrane glycoprotein. The c-*erb*B-2 and EGF receptor possess transmembrane spanning sequences and have homologous intracellular domains associated with a tyrosine kinase activity (Akiyama *et al.*, 1986). However, down modulation of c-*erb*B-2 expression is not triggered by the addition of EGF in biological assays and it is proposed that this putative receptor interacts with an, as yet, unidentified ligand product. Although the EGF and c-*erb*B-2 receptors are closely related from sequence data, they are localized to different chromosomes (7 to 17 respectively, Schechter *et al.*, 1985) and each can be found amplified in the presence of a single copy of the other. Recent studies have implicated an interaction between these two families via receptor phosphorylation following ligand binding (Kokai *et al.*, 1988; Bargmann & Weinberg, 1988).

Amplification of proto-oncogenes, leading to the over-expression of the protein product, has been demonstrated to lead to transformation in a number of cell systems and has recently been reported for c-*erb*B-2 in NIH3T3 cells (Hudziak *et al.*, 1987; DiFiore *et al.*, 1987). Increased expression of this proto-oncogene product has gained clinical interest from the observation that amplification of this gene in breast carcinomas may be related to poor prognosis (Slamon *et al.*, 1987). Further studies have confirmed the relatively high frequency of amplification (Zhou *et al.*, 1987) and demonstrated a correlation with elevated levels of expression of the p185 protein product in corresponding tissue samples (Venter *et al.*, 1987; Berger *et al.*, 1987; Van de Vijer *et al.*, 1988). Included in these studies is a survey of DNA isolated from a variety of adenocarcinomas which revealed amplification of c-*erb*B-2 in only a small number of cases including stomach (Yokota *et al.*, 1986; Fukushige *et al.*, 1986; Tal *et al.*, 1988), kidney (Yokota *et al.*, 1986), salivary gland (Semba *et al.*, 1985) and colon tumors (Tal *et al.*, 1988). Although multiple copies of the c-*erb*B-2 gene have been found infrequently in colon carcinomas, studies on breast carcinoma cell lines have revealed elevated levels of expression of the 185 kD protein in the absence of gene amplification or rearrangement (Kraus *et al.*, 1987). This has prompted us to study the expression of this putative receptor in human colon tissue representing different stages of neoplastic progression using antibodies which have been previously shown to identify the p185 product in paraffin embedded tissue sections (Gullick *et al.*, 1987). This paper reports the occurrence of multiple copies of the c-*erb*B-2 gene in one colonic polyp and three carcinomas and the expression of the 185 kD in normal, benign and malignant human colonic tissue.

Results

Gene amplification in primary colon lesions

DNA from five polyps and forty-four primary human colon carcinomas was isolated from frozen tissue sections of tumor material and probed in Southern blot analysis using a c-erbB-2 cDNA probe (Yamamoto *et*

Correspondence: I.C. Summerhayes, New England Deaconess Hospital, Laboratory of Cancer Biology, 50 Binney Street, Boston, MA 02115, USA
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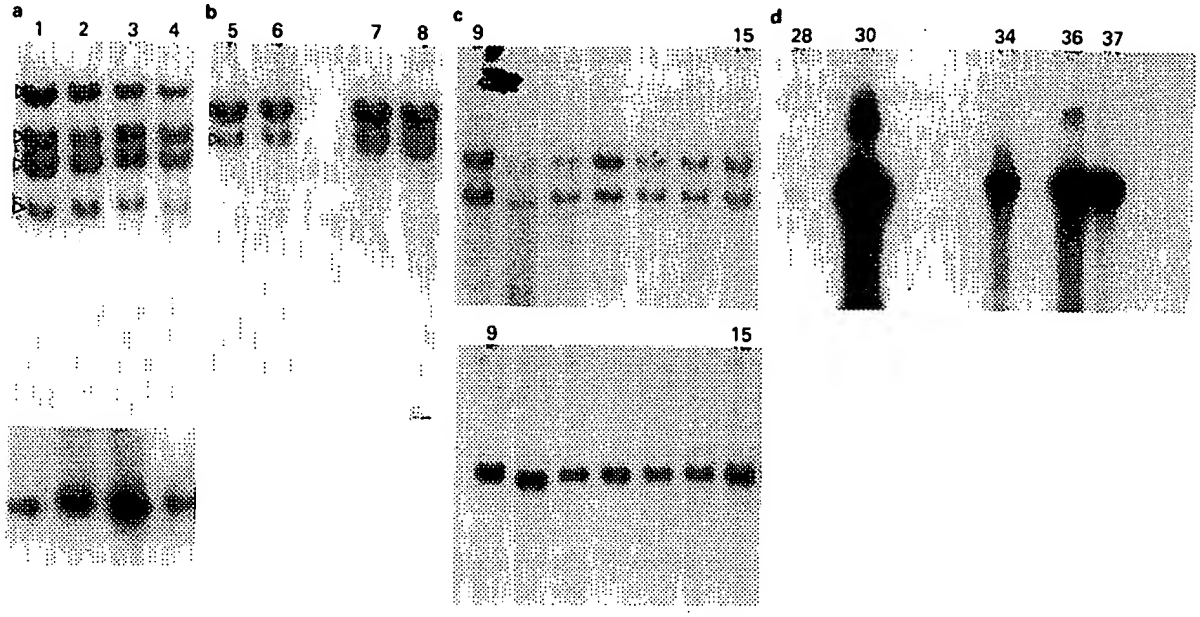

Figure 1 Southern blot analysis of the c-*erb*B-2 gene in primary polyp and tumor DNA. Ten micrograms of tumor DNA was digested with EcoRI (Figure 1a) or HindIII (Figure 1b–1d) and hybridized with a 3.0 kb HindIII-KpnI fragment containing c-erbB-2 cDNA specific sequences. Following hybridization, the probe was removed and the blot was rehybridized with the α-2-1 collagen specific probe to standardize DNA loading (lower panel in 1a and 1c). Only a representative sample of tumors are shown with one blot (Figure 1d) containing the four DNA's harboring multiple c-*erb*B-2 gene copies. Figure 1a–EcoRI digested colon tumor DNA; Figure 1b–1d HindIII digested colon tumor DNA. Figure 1c represents a HindIII digest of tumors 9–15 showing a single copy level of the c-*erb*B-2 gene; lower panel is the same blot probed with the α-2-1 collagen probe. Figure 1d represents a short exposure of a gel containing tumor DNA's with multiple c-*erb*B-2 gene copies (tumors 30, 34, 36, 37). The larger size restriction fragment at approximately 14 kilobases is evident in all samples following longer exposure of the gel. Lane 28 is SK-BR-3 DNA included as a positive control; lanes 30, 34, 36 are DNA from separate colonic adenocarcinomas and lane 37 is DNA isolated form a polyp. The remaining lanes include tumor DNA with a single copy level of the gene where restriction fragments were resolved on longer exposure

al., 1986). Figure 1a shows a representative panel of DNA from colon tumors digested with the restriction endonuclease EcoRI where the bands generated were characteristic of the sizes previously reported for this gene (Yokota *et al.*, 1986). All tumor DNA's were probed following digestion with EcoRI or HindIII (Figures 1a and 1b respectively) and no major rearrangement of the c-*erb*B-2 gene was detected by Southern blot analysis. Blots were stripped and re-probed with the α-2-1 collagen specific probe indicating relative single gene copy levels of c-*erb*B-2 (Figure 1c). One of five cases of DNA isolated from polyp lesions and in three carcinomas, amplified copies of the c-*erb*B-2 gene were detected (Figure 1d) and confirmed by re-probing blots with the α-2-1 collagen control probe. Figure 1d shows a short exposure of a Southern blot containing the three tumor (Figure 1d lanes 30, 34, and 36) and one polyp DNAs (Figure 1d lane 37) with multiple c-*erb*B-2 gene copies, showing amplification relative to SK-BR-3 (Figure 1d lane 28) which harbors approximately 10–15 copies of this gene. The larger size 14 kilobase restriction fragment, generated following HindIII digestion, can be resolved in all cases (Figure 1d) after longer exposure of the gel. Although amplification was evident in DNA samples prepared from unscreened tumor material, this was greatly enhanced when the same tissue was evaluated after sectioning to remove stromal elements, with DNA preparation from tissue sections.

Antibody specificity and c-erbB-2 expression in colon carcinoma cell lines

A series of anti-peptide antibodies, which immunoprecipitate the c-erbB-2 gene product have been described and shown not to cross react with the closely related EGF receptor (Gullick *et al.*, 1987). Two antibodies, designated 20N and 21N, were raised to residues 1215–1225 or 1243–1255 respectively of the c-*erb*B-2 open reading frame and have subsequently been used on a series of breast carcinomas where over-expression of the c-erbB-2 gene product is relatively frequent (Venter *et al.*, 1987). From such studies the staining observed, in both frozen and paraffin embedded sections, has been shown to correlate with the expression of the p185 protein, given the gene copy number established from each tumor DNA. Confirmation of the specificity of both antibodies was established in both immunoprecipitation and Western blot analysis using a panel of human colorectal cell lines with the breast carcinoma cell line, SK-BR-3, as a control in these experiments (Figure 2). Immunoprecipitation of cell lysates from ten established colon cell lines, following an 18 h incubation with [^{35}S]cysteine or [^{32}P]orthophosphate, revealed precipitation of a single band at 185 kD from SK-BR-3 with little evidence of this protein in the colon carcinoma cell lines studied (Table 1). In only three cell lines could we detect the presence of the 185 kD protein product. Figure 2a shows immunoprecipitation of the c-erbB-2 protein from [^{32}P]-labeled cell lysates of the control cell line SK-BR-3 (lane 1), a colon cell line expressing the protein (lane 2) and one where the putative receptor was not found (lane 3). Similar results were obtained with both 20N and 21N antibodies and their specificity was maintained when the same cell lines were analysed in Western blots (Figure 2b).

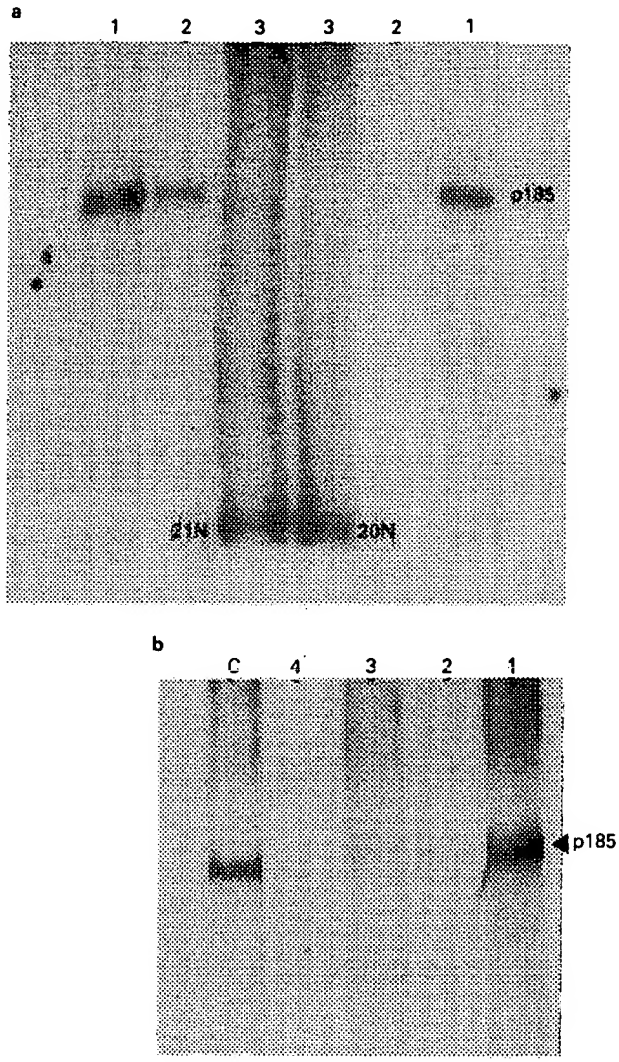

Figure 2 (a) Immunoprecipitation of the p185 c-erbB-2 protein from [^{32}P]orthophosphate-labeled cell lysates using antibodies 20N and 21N. Lane 1 SK-BR-3, human mammary carcinoma cell line; Lane 2 SW403 colon adenocarcinoma cell line; Lane 3 SW480 colon adenocarcinoma cell line. (b) Western blot analysis of total cell lysates from colon tumor derived lines, probed with the 20N antibody. Lane 1 SW403; Lane 2 CX-1; Lane 3 SW837; Lane 4 SW480; Lane C represents positive control NIH3T3 cells expressing a truncated c-erbB-2 protein

Expression of the c-erbB-2 product in normal colon

To establish a baseline for immunocytochemical observations we have used a series of ten colon specimens taken from individuals with no known colon disease. In all cases both 20N and 21N antibodies, used in separate experiments, showed a staining distribution localized to the luminal surface cells of the colon with no crypt involvement (Figure 3). The staining observed was associated with both cytoplasm and membrane, with the basolateral region of the apical cells staining most strongly. This staining was not observed when pre-immune serum was substituted for the first antibody and could be competed out by pre-incubation of the peptide antibody with the appropriate peptide (data not shown). In all ten cases the staining observed did not involve cells in the crypt.

Figure 3 Expression of p185 in normal colon epithelium localized to cells at the luminal surface with an absence of staining in cells of the crypt (×350)

Expression in colon carcinoma tissue

In a series of forty carcinomas, representing a range of Duke stages, only eight tumors showed unequivocal staining with both antibodies. This group included two of the tumors identified as harboring multiple copies of the c-*erb*B-2 gene, both of which showed intense staining with 20N and 21N antibodies. The staining pattern observed was represented by an intense granular appearance at cytoplasmic and membrane locations (Figure 4a). In all cases, staining was competed out by the appropriate peptide in control experiments. A number of other tumor sections revealed a faint cytoplasmic staining, which in contrast to the granular staining, was often lost on further dilution of the primary antibody. Interestingly, in twelve tumor sections, negative for staining with either antibody, adjacent areas of histologically normal mucosa were present which showed intense staining with these antibodies (Figure 4b). In most of these instances localization of the c-erbB-2 protein extended into the cells of the crypt (Figure 4c), in contrast to our observations of normal colonic mucosa from disease-free individuals (compare Figures 3 and 4c). These histologically normal areas, adjacent to tumors, served as an internal control in sections where carcinomatous material showed an absence of c-*erb*B-2 expression.

The more extensive expression of c-*erb*B-2 in 'normal' tissue adjacent to tumors may indicate alterations associated with compromised mucosal elements in this region. To investigate the range of this altered field we have taken adjacent normal colon from measured

Table 1 Expression of p185 in human colonic polyps

	No. studied	*No. staining**	*Comments*
Tubular adenomas	19	18	Staining throughout crypt structures
Tubulo-villous adenomas	16	11	3/5 not staining showed severe dysplasia
Villous adenomas	2	1	Focal staining in positive specimen†

* Staining was assessed using 20N and 21N antibodies in repeated experiments
† Focal staining associated with cells of the luminal polyp surface

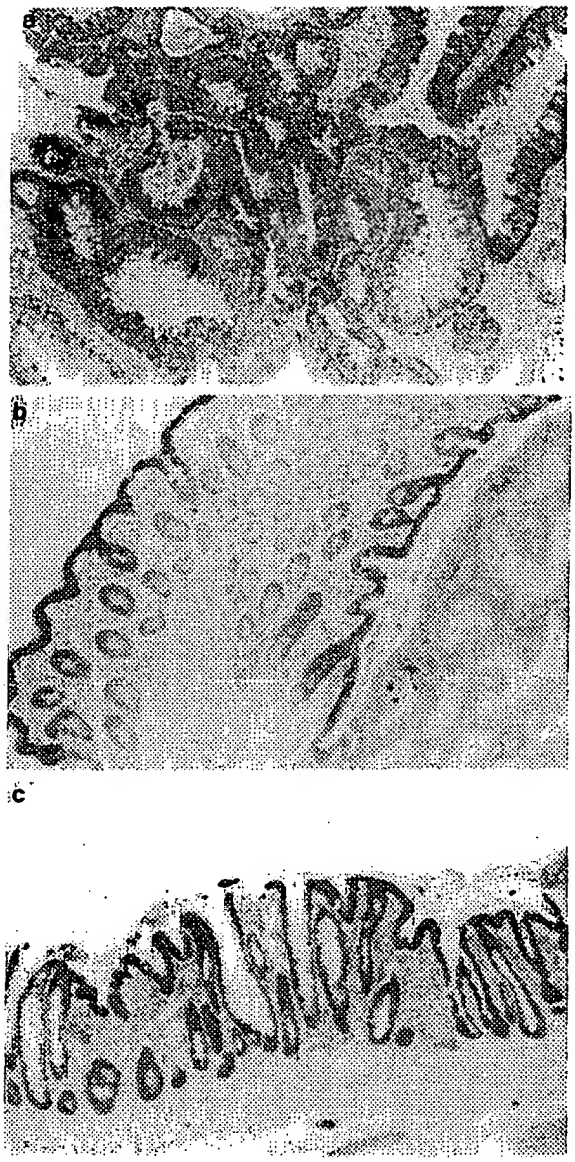

Figure 4 Immunocytochemical staining of different representative colonic lesions following incubation with 20N or 21N antibodies. (a) Moderate to well differentiated colon adenocarcinoma (× 300); (b) Normal colonic mucosa, adjacent to a neoplastic lesion which revealed no detectable p185 expression in immunostaining. Note the localized expression of p185 at the luminal surface in apparently normal mucosa (× 125). (c) Section characteristic of mucosa adjacent to a colonic tumor showing extended distribution of p185 into cells of the crypt (× 125)

intervals both proximal and distal to the tumor site for immunocytochemical study. Mucosa proximal to the tumor at intervals of 5–15 centimeters displayed solely luminal staining in 12/13 specimens with no crypt involvement. Similarly, mucosa distal t the tumor, taken at the same intervals, showed normal luminal staining in 12/14 specimens. In all cases studied the normal mucosa immediately adjacent to the tumor displayed an aberrant distribution of c-*erb*B-2 involving cells deep into the crypt, however, this was maintained in only two cases, five centimeters from the neoplastic lesion.

*c-*erb*B-2 expression in premalignant polyps*

Neoplastic progression in the colon is characterized by the appearance of preneoplastic polyps, defined histologically as tubular, tubulo-villous or villous adenomas (Table 1). This study was extended to include sections from thirty-seven such lesions, however, in only five cases were we able to obtain DNA from the samples for analysis in Southern blots. Twenty-nine of thirty-seven polyps showed some staining with both 20N and 21N antibodies (Table 1), either a diffuse cytoplasmic stain (14 cases) or the granular cytoplasmic/membrane staining observed in carcinomas (23 cases). Sections from polyps harboring multiple copies of the c-*erb*B-2 gene showed a granular cytoplasmic/membrane staining (Figure 5) pattern which was the criterium used for positive expression of the c-erbB-2 product in this study. In all cases, staining was competed out by preincubation of the antibody with the appropriate peptide. Confirmation of the presence of the p185 product in tumor and polyp material was established in Western blot analysis, probing lysates with the 20N antibody (Figure 6). Although the background staining was much higher when using tumor tissue preparations the inclusion of tumors, consistently negative in immunocytochemical staining, distinguished the presence of the p185 protein in both polyp and tumor tissue analysed. In a number of polyp sections, displaying a

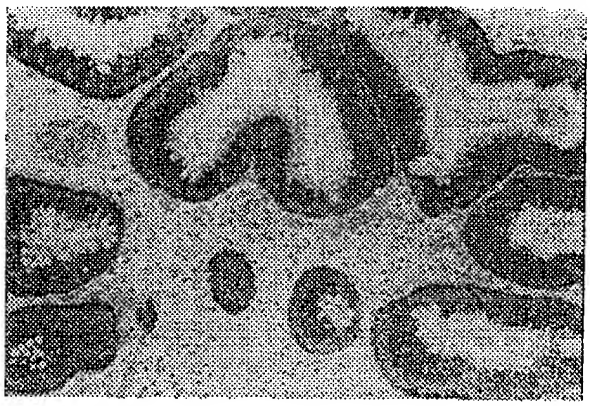

Figure 5 Tubular adenoma showing expression of the p185 product in cytoplasmic and membrane locations (× 300)

Table 2 Expression of p185 in human colorectal cell lines

Human cell lines		*Derivation/Differentiation*	*Expression*
DLD-2		Colon adenocarcinoma/moderately differentiated	–
SW837	(CCL 235)	Rectal adenocarcinoma/grade IV	+
SW403	(CCL 230)	Colon adenocarcinoma/grade III	+
SW 480	(CCL 228)	Colon adenocarcinoma/grade III–IV	–
COLO 320 DM	(CCL 220)	Colon adenocarcinoma/moderately undifferentiated	–
COLO 320 HSR	(CCL 220.1)	Colon adenocarcinoma/subclone of Colo 320	–
SW620	(CCL 227)	Colon adenocarcinoma/lymph node metastasis	–
LoVo	(CCL 229)	Colon adenocarcinoma/lymph node metastasis	–
MIP 101		Colon carcinoma/poorly differentiated	–
Clone A		Colon carcinoma/poorly differentiated	+

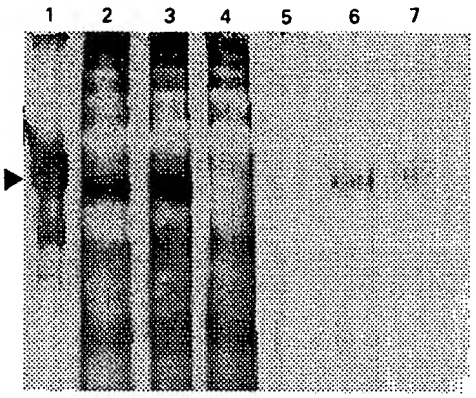

Figure 6 Western blot analysis of total cell lysates from tumor and polyp material probed with the 20N antibody. The tumor material in lanes 2 and 3 displayed expression of p185 in immunocytochemical studies whilst lane 4 represents a carcinoma negative in staining. Lane 1 prestained 200000 dalton molecular weight marker (arrow); Lane 2 moderate-well differentiated colon carcinoma; Lane 3 tubular adenoma; Lane 4 moderate-well differentiated colon carcinoma; Lane 5 NIH3T3 cell lysates; Lane 6 NIH3T3 transfectants expressing the c-erbB-2 protein; Lane 7 SK-BR-3 mammary carcinoma cell line

gradation of pathological features ranging from normal to dysplastic, heterogeneous staining was observed. The most intense staining was observed in areas of benign morphology with a less intense, or absence of staining in dysplastic regions within the same polyp.

Discussion

In this paper we report the localization of the c-erbB-2 gene product in normal human colon and in tissue representing different stages of neoplastic progression. Multiple copies of the c-*erb*B-2 gene have been reported in a number of adenocarcinomatous lesions, particularly mammary carcinomas, where the resulting elevated levels of expression are considered a primary event in neoplasia, an interpretation supported by recent work using transgenic mice harboring the neu oncogene (Muller *et al.*, 1988). Other investigations have shown that although elevated levels of expression of the c-erbB-2 protein are often associated with gene amplification, this is not always the case (Bargmann *et al.*, 1986; Kraus *et al.*, 1987). The objective in this study was to evaluate the expression of the c-*erb*B-2 185 kD protein in different colonic lesions, against the molecular background of the gene copy number.

Our study demonstrates that amplification of the c-*erb*B-2 gene is an infrequent event in colon tumors (6.8%), consistent with the previously published reports (Yokota *et al.*, 1986; Tal *et al.*, 1988). In addition we report one case of DNA from preneoplastic polyps where multiple copies of the c-*erb*B-2 gene were detected, however, the sample number (1/5) is too small to comment on the significance of this finding. We did not find evidence of rearrangement of the c-*erb*B-2 gene in the tumor tissue studied. Although the preneoplastic polyps analysed at the gene level are few, due to the limited material available, immunocytochemical staining of sections revealed increased levels of expression of the p185 protein in $>60\%$ of these lesions. This observation was confirmed in Western blot analysis. In contrast only 20% of carcinomas showed staining with both 20N and 21N antiserum, indicative of a reduced expression of p185 associated with progression to malignancy. This correlation was also suggested within polyp lesions, where different regions within the polyp displayed a heterogeneous pathology with more dysplastic areas displaying an absence of staining. In addition the aberrant distribution of p185 observed in 'mormal' mucosa, adjacent to tumor material, is suggestive of altered c-*erb*B-2 expression associated with compromised colonic mucosal elements. It is not clear whether this represents a premalignant change or a hyperproliferative state and it will be of interest to study colonic tissue taken from polyposis families, at both early and late stages of progression. In any event the first indication of altered expression of the p185 protein in normal tissue was that observed adjacent to the tumor, a phenotype retained in preneoplastic polyp lesions.

The c-erbB-2 gene product exhibits close homology to the epidermal growth factor receptor although they are localized to different chromosomes within the human genome (Schechter *et al.*, 1985). It is interesting to note that deletions associated with chromosome 17 have been reported in 76% of colon carcinomas, although they are restricted to the short arm of chromosome 17 and are not observed at a high frequency in adenomatous polyps (Vogelstein *et al.*, 1988). These mapped deletions do not involve the c-*erb*B-2 gene, which is localized to the long arm of chromosome 17 (17q 11.2-q22), but demonstrate that this chromosome is a target for change in colonic neoplastic progression. The putative function of the c-erbB-2 protein product, as a growth factor receptor, is deduced from its homology to other known growth factors, but as yet lacks an identified ligand. Restricted localization of the protein to the luminal cells of normal colon epithelium demonstrates an association with the fully differentiated epithelial cells, since no staining is observed in cells of the proliferative compartment of the crypt. This distribution was also observed in rat colon epithelium using the same antibodies and contrasts with that recently reported by Cohen *et al.* (1989) where they reported c-*erb*B-2 expression throughout all cells of the mucosa. The antibody used in their study was raised against a synthetic peptide that spans the peptide region used for the immunogen in raising the antibody 21N used in this study (Gullick *et al.*, 1987). Although our overall findings are similar with regards to c-*erb*B-2 expression in preneoplastic lesions and established cell lines, we find fewer tumors positive for staining and a more restricted localization of p185 in normal mucosa. It seems unlikely that the use of an additional three amino acid residues in the immunogen peptide would account for this difference and more likely that the fixation or tissue preparation account for this. In addition we observed no stromal staining associated with the use of 20N or 21N antibodies in most tissue sections and could clearly define the membrane associated staining previously characterized in breast tumors with these antibodies (Barnes *et al.*, 1988; Gusterson *et al.*, 1988).

Whether the altered levels of expression of the c-erbB-2 gene product reflect a primary event in the generation of polyps is not clear, however, the finding of extensive amplification of this gene in four colonic lesions suggests a potential role for this gene via the route proposed for breast carcinomas. The reduced levels of p185 found in tumor sections is consistent with

the immunoprecipitation data obtained using tumorigenic colon cell lines. Presently we need to extend our work on the preneoplastic polyps to establish whether the observations reported, have a potential clinical significance toward identifying high risk polyps likely to progress to carcinomas.

Materials and methods

Cells

Colorectal cell lines were obtained from ATCC, and are routinely passaged in Dulbecco's Modified Eagles Medium/RPMI (50/50) supplemented with 5% calf serum 5% fetal bovine serum. The human mammary carcinoma cell line SK-BR-3 was used as a positive control in all experiments involving Southern blot analysis and immunoprecipitation of the c-erbB-2 protein product.

Isolation of DNA

Since many colon tumors contain extensive mesenchymal components, we have prepared DNA from lesions by the method of Vogelstein *et al.* (1988). Tissue samples were frozen in OCT embedding compound (Miles Scientific) and a cryostat section was prepared and evaluated with respect to the distribution of neoplastic elements within the tissue specimen. Regions of the tissue representing a majority of neoplastic cells were excised from frozen block and re-embedded in OCT compound. A second section was prepared from this tissue to confirm the original evaluation. Sections were cut to a total of 200 with numbers 51, 101, 151 being processed in histological staining to monitor the continued neoplastic nature of the tissue. In appropriate cases the 200 sections were pooled and DNA was extracted following an overnight incubation in proteinase K and 1% SDS. All DNA isolates were harvested by pooling and checked in agarose gels to confirm the high molecular weight nature of DNA preparations.

Immunoprecipitation and Western blot procedures

Antibodies 20N and 21N were a generous gift from Dr W. Gullick (Gullick *et al.*, 1987). Cells used in immunoprecipitation were incubated for 18 h in the presence of [^{35}S]cysteine (100 μCi ml^{-1}) or [^{32}P]orthophosphate and immunoprecipitates and Western blot analysis were performed as previously described (Gullick *et al.*, 1987). In the case of radiolabeled cells, samples were analysed on a 7.5% polyacrylamide gel containing SDS, stained with Coomassie blue and autoradiographed at −70°C for 1–5 days. In each experiment, the human mammary cell line SK-BR-3 was run as a positive control. In Western blot analysis, confirmation of the specificity of staining was established by peptide competition in each experiment. Antibody 20N was found to be superior for use in Western blot analysis.

Southern blots

DNA's were digested under conditions recommended by the manufacturer using a 10-fold excess of enzyme. Digested DNA's were electrophoresed in 0.8% agarose gels and transferred to the nylon membrane Hybond-N. Hybridization was carried out at 42°C in a buffer containing 50% formamide and 10^6 cpm ml^{-1} random primed probe. The probe used for recognition of c-*erb*B-2 gene was a 3.0 kilobase HindIII-KpnI fragment of human c-erbB-2 complementary DNA clone pCER 204 (Yokota *et al.*, 1986). Control probe used to standardize DNA leading in these experiments was the α-2-1 collagen-specific probe (Venter *et al.*, 1987).

Immunocytochemical staining

Sections (5 μM) were cut from formalin-fixed, paraffin embedded tissue, deparaffinized and rehydrated. Endogenous peroxidase activity was destroyed by a 30 min treatment with hydrogen peroxide methanol solution followed by extensive washes in phosphate buffered saline (PBS). Non-specific protein binding was blocked by a 30 min incubation with non-immune goat serum followed by a brief wash in PBS. Affinity purified peptide antibodies (20N or 21N) were applied to the section (20 μg ml^{-1}) and incubated overnight at 4°C. In separate experiments staining with either antibody was shown to be competed out by the appropriate peptide. In cases of peptide competition, 25 μg of the peptide was added to 100 μl of the diluted antibody and incubated for 30 min at room temperature. Following incubation with the antibody or peptide incubated preparation, sections were washed in PBS and incubated for 10 min at room temperature with 50 μl of a biotinylated second antibody, goat anti-rabbit (Zymed histostain-sp kit, S.F.) followed by the enzyme conjugate and a substrate-chromogen mixture. Slides were counterstained with hematoxylin, rinsed in distilled water and mounted for viewing.

Acknowledgements

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L5 ANSWER 2 OF 5 CANCERLIT
AN 95611685 CANCERLIT
DN 95611685
TI Monoclonal **antibodies** directed against growth factor receptors enhance the efficacy of **chemotherapeutic** agents (Meeting abstract).
AU Baselga J; Fan Z; Norton L; Mendelsohn J
CS Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
SO Ann Oncol, (1994) 5 (Suppl 5) A010.
ISSN: 0923-7534.
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Institute for Cell and Developmental Biology
EM 199509
ED Entered STN: 19950906
Last Updated on STN: 19950906
AB Several lines of evidence suggest that growth factor receptor blockade may enhance the antitumor effects of **chemotherapeutic** agents. We have produced monoclonal **antibodies** (MAbs) directed against the epidermal growth factor receptor (EGFR). MAbs 225 (IgG1) and 528 (IgG2a) are capable of preventing growth of subcutaneous human tumor xenografts in nude mice when treatment is started shortly after tumor cell inoculation, but they are only moderately inhibitory after tumors are well developed. Therefore, we have explored combination treatment with **chemotherapeutic** agents plus anti-EGFR MAbs. Three drugs have been studied: doxorubicin, paclitaxel (Taxol) and cisplatin. Treatment of well established (greater than 300 mm3) A431 squamous carcinoma xenografts with maximally tolerated doses of doxorubicin (5 mg/kg ip for two successive days) had weak antitumor activity, whereas treatment in combination with either MAbs 528 or 225 at a dose level of 1 mg ip twice a week or higher resulted in eradication of tumors in 40 to 69% of the animals in several independently conducted experiments. Similar results were obtained when the same combination was used in well developed (greater than 200 mm3) MDA-468 breast adenocarcinoma xenografts. In subsequent studies, well developed MDA-468 nude mice xenografts were treated with paclitaxel (10-20 mg/kg weight iv x 2) in combination with MAb 528 2 mg intraperitoneally (ip) twice a week for ten doses. There was striking inhibition of growth compared with animals treated with either paclitaxel or MAb 528 alone. Studies with cisplatin in well established A431 cell xenografts at a maximally tolerated dose of 6 mg/kg x 2 in combination with MAb 225 have also resulted in eradication of xenografts resistant to drug alone. We then analyzed the effects of doxorubicin and paclitaxel in combination with MAb 4D5 directed against the **HER2** receptor. BT-474 breast adenocarcinoma cells, which express high levels of **HER2**, were grown to a mean size of 200 mm3. A dose of 4D5 that resulted in a modest inhibition of growth was chosen for these studies. Treatment with anti-**HER2** MAb 4D5 alone (0.3 mg/kg ip twice a week) produced 35% growth inhibition, paclitaxel alone (20 mg/kg iv) resulted in 35% inhibition and doxorubicin alone (10 mg/kg ip) in 27% inhibition of growth. The combined treatment with paclitaxel plus 4D5 resulted in a major antitumor activity with 93% inhibition of growth, and doxorubicin plus 4D5 gave 70% inhibition. Studies of cisplatin in combination with 4D5 are underway. In all of the studies, MAbs did not increase the toxicity of the **chemotherapeutic** agents in animals as determined by animal survival and weight loss. Clinical trials that build on these results are planned. In summary, anti-EGFR MAbs markedly enhance the antitumor effects of the **chemotherapeutic** agents doxorubicin, paclitaxel and cisplatin. Anti-**HER2** MAb also enhances the antitumor effects of doxorubicin and more significantly of paclitaxel. Studies of mechanisms are underway.
RN 15663-27-1 (Cisplatin); 23214-92-8 (Doxorubicin); 33069-62-4 (Paclitaxel)
CN 0 (**Antibodies**, Monoclonal); 0 (Receptors, Growth Factor)

HER2 (c-erbB-2) oncoprotein expression in colorectal adenocarcinoma: an immunohistological study using three different antibodies.

Arnaout AH, Dawson PM, Soomro S, Taylor P, Theodorou NA, Feldmann M, Fendly BM, Shepard HM, Shousha S.

Department of Histopathology, Charing Cross Hospital, Charing Cross and Westminster Medical School, London.

Paraffin wax sections of 70 surgically resected colorectal adenocarcinomas were examined for the overexpression of HER2/c-erbB-2 oncoprotein using three different specific antibodies and the avidin-biotin immunoperoxidase technique. The patients included 38 men and 32 women aged between 47 and 80 years. The tumours were derived from various parts of the large intestinal tract, and represented all three stages of Dukes' classification and the three histological grades of differentiation. Many tumour sections also included adjacent normal or transitional mucosa. Eight tubular adenomas found in the colectomy specimens in association with some carcinomas were also examined. No positive membrane staining was seen in any of the 70 carcinomas, four adenomas, two hyperplastic polyps, nor in the adjacent normal or transitional mucosa. It is suggested that the overexpression of c-erbB-2 gene product is unlikely to be as common and as pronounced in colorectal adenocarcinoma as it is in ductal carcinoma of the breast.

PMID: 1357006 [PubMed - indexed for MEDLINE]

1: Growth Regul 1991 Jun;1(2):72-82

Characterization of an anti-p185HER2 monoclonal antibody that stimulates receptor function and inhibits tumor cell growth.

Sarup JC, Johnson RM, King KL, Fendly BM, Lipari MT, Napier MA, Ullrich A, Shepard HM.

Department of Developmental Biology, Genentech, Inc., South San Francisco, California 94080.

The HER2 protooncogene encodes a growth factor receptor-like transmembrane protein tyrosine kinase (p185HER2) whose ligand remains to be fully characterized. The overexpression of p185HER2 is implicated in aggressive forms of breast and ovarian cancers. The role of p185HER2 in aggressive malignancy, as well as its cell surface localization, makes it an attractive target for therapeutic monoclonal antibodies. In this report we have studied the modulation of p185HER2 function with 2 monoclonal antibodies, termed 4D5 and 6E9, which bind the extracellular domain of p185HER2. 4D5 inhibited proliferation of p185HER2 overexpressing SK-BR-3 human breast carcinoma cells (ED50 of approximately 1 nM) but did not inhibit proliferation of cultured human breast carcinoma MCF7 cells, low expressors of p185HER2. Monoclonal antibody 6E9 does not inhibit the growth of either cell line. Antibody binding studies revealed 2 populations of p185HER2 molecules on SK-BR-3 cells: one of high abundance (approximately 2 x 10(6) sites/cell) recognized by 4D5 (Kd approximately 6 nM) and the other of low abundance (2 x 10(4) sites/cell) recognized by 6E9 (Kd approximately 0.1 nM). 4D5, in an agonistic manner, downregulated SK-BR-3 cell surface p185HER2, was internalized, and stimulated p185HER2 phosphorylation in intact cells. Phosphoamino acid analysis of p185HER2 derived from SK-BR-3 cells incubated with the 4D5 monoclonal antibody demonstrated increased tyrosine, serine and threonine phosphorylation. 4D5, on short term (5 min) exposure to SK-BR-3 cells, stimulated inositol lipid hydrolysis as evidenced by increased intracellular levels of inositol polyphosphates (InsP) and sn-1,2-diacylglycerol (sn-1,2-DAG). On longer (24 h) exposure to the cells, the antibody appeared to downregulate this signalling pathway since the intracellular levels of InsP and sn-1,2-DAG decreased by 30 to 40%. 6E9 did not inhibit SK-BR-3 cell proliferation, downregulate surface p185HER2, stimulate receptor

phosphorylation, or stimulate the second messenger pathway. Despite these agonistic properties, 4D5 was an inhibitor of SK-BR-3 cell proliferation at all concentrations tested (0.7 to 70 pM). The data suggest that 4D5 is a partial or weak agonist and thus may inhibit cell proliferation by mimicking ligand-like receptor downregulation.

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1: J Clin Immunol 1991 May;11(3):117-27

Monoclonal antibody therapy of human cancer: taking the HER2 protooncogene to the clinic.

Shepard HM, Lewis GD, Sarup JC, Fendly BM, Maneval D, Mordenti J, Figari I, Kotts CE, Palladino MA Jr, Ullrich A, et al.

Department of Developmental Biology, Genentech, Inc., South San Francisco, California 94080.

The HER2 protooncogene encodes a 185-kDa transmembrane protein (p185HER2) with extensive homology to the epidermal growth factor (EGF) receptor. Clinical and experimental evidence supports a role for overexpression of the HER2 protooncogene in the progression of human breast, ovarian, and non-small cell lung carcinoma. These data also support the hypothesis that p185HER2 present on the surface of overexpressing tumor cells may be a good target for receptor-targeted therapeutics. The anti-p185HER2 murine monoclonal antibody (muMAb) 4D5 is one of over 100 monoclonals that was derived following immunization of mice with cells overexpressing p185HER2. The monoclonal antibody is directed at the extracellular (ligand binding) domain of this receptor tyrosine kinase and presumably has its effect as a result of modulating receptor function. In vitro assays have shown that muMAb 4D5 can specifically inhibit the growth of tumor cells only when they overexpress the HER2 protooncogene. MuMAb 4D5 has also been shown to enhance the TNF-alpha sensitivity of breast tumor cells that overexpress this protooncogene. Relevant to its clinical application, muMAb 4D5 may enhance the sensitivity of p185HER2-overexpressing tumor cells to cisplatin, a chemotherapeutic drug often used in the treatment of ovarian cancer. In vivo assays with a nude mouse model have shown that the monoclonal antibody can localize at the tumor site and can inhibit the growth of human tumor xenografts which overexpress p185HER2. Modulation of p185HER2 activity by muMAb 4D5 can therefore reverse many of the properties associated with tumor progression mediated by this putative growth factor receptor. Together with the demonstrated activity of muMAb 4D5 in nude mouse models, these results support the clinical application of muMAb 4D5 for therapy of human cancers characterized by the overexpression of p185HER2.

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